Does the Tyndall Effect Describe the Blue Hue Periodically Observed in Subdermal Hyaluronic Acid Gel Placement?

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Background The blue hue of skin overlying injected hyaluronic acid (HA) fillers in certain cases has been hypothesized in the literature as related to the Tyndall effect. This investigation aims to understand the relevant optical concepts and to discuss the plausibility of this assertion.

Methods: Theoretic and physical aspects of relevant optical theories including the Tyndall effect, the Raleigh criterion and the Mie Solution are discussed, with simple examples. The physical properties of the system (both HA and subcutaneous tissue) are explored. Alternate concepts of dermal hue generation are discussed.

Results: The Tyndall effect (and Rayleigh criterion) describe optical phenomenon that occur as light passes through colloidal solutions containing uniform spherical particles of sizes less than the length of a wavelength of visible light. HA fillers are complex, large, non-spherical, cross-linked hydrogels, and thus are not well characterized by these theories.

Skin is a complex optical surface in which shorter wavelengths of light are selectively filtered at superficial depths. Light passing through to subdermal HA would have low blue light amplitude, minimizing what light could be preferentially scattered. Further, should blue hues be 'generated' subdermally, the same skin filters work in reverse, making the blue light poorly detectable by an external observer.

Conclusions: The Tyndall effect is unlikely to cause dermal hue changes in HA filler instillation. Optical and perceptual processes explaining superficial vein coloration may better describe subdermal HA hue changes. Vein coloration is thought to be related to three processes: the reflective properties of the skin, the absorptive properties of blood and the perceptive properties of an observer's eyes. Subdermal HA may simulate these phenomena by a number of undetermined, yet plausible mechanisms.

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Hyaluronic acid (HA) gel fillers are widely used for cosmetic rejuvenation of the periocular region, with mostly good effect.[1] They are extremely safe to use overall, with low complication rates. However, they are not completely without side effects, and one of the more common and least understood of these is the appearance of a blue hue to the skin overlying a region after filling.[2,3]

As originally described by Hirsch et al.,[4,5] this blue hue has been thought optically to be caused by light scattering phenomena known as the Tyndall effect. Hirsch et al. suggest that the blue color in HA gel injection can be explained in much the same way as the blue sky, in that shorter wavelengths are scattered preferentially, creating a blue color perceived by an observer's eyes. However, the physical optics of this phenomenon is not elaborated in these publications. Despite a lack of theoretical or experimental data, the moniker of "Tyndall effect" has persisted.[6]

The purpose of the present discussion is to describe the relevant optical principles governing the Tyndall effect and the Raleigh criterion as they apply to the HA gel problem and to discuss alternative optical explanations for the blue hue phenomenon.

THE TYNDALL EFFECT, THE RALEIGH CRITERION, AND THE MIE SOLUTION

The nineteenth century physicist John Tyndall (August 2, 1820,to December 4, 1893) made the astute observation that a cone of white daylight can be seen as it passes through a window into a dark room. Living in postindustrial England where the air was thick with particulate matter, he reasoned that light was reflecting off molecules in the air, making them visible to the human eye. He further found that some types of airborne particles created a blue tinge to the cone of light and postulated that this same phenomenon was responsible for the blue color of the sky.[7] This phenomenon has since dubbed the Tyndall effect or the Tyndall cone.

As an aside, these observations led to seminal works providing evidence for germ theory. Tyndall later demonstrated in a series of experiments that living organisms were found among these particles. He found that pasteurized foods contained in boxes with purified air (that did not demonstrate Tyndall scattering) did not decompose. While the same foods in boxes with particulate filled air, swarm with life within a few days.[8]

Building from these seminal observations, his successor John William Strutt, the third Baron Rayleigh, (November 12, 1842, to June 30, 1919) was able to provide mathematical and experimental proof of this theory. Lord Raleigh understood these scattering effects to be the result of interactions between light and molecular-level electrical fields. His experiments and equations characterized these systems, and suggested that the intensity of scattering is inversely proportional to the fourth power of the wavelength of light. In his solution, blue light is scattered 10× more intensely than red.[9]

Importantly however, this conceptualization presumes that the wave of light is affected uniformly by the particle's electrical field, and for this to be true, the particle size must be much smaller

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than the wavelength. Thus, Raleigh's approximations generally hold only for particles less than 1/10 of the size of a wavelength of visible light or about 40 nm (atomic-sized particles). His theorem proved Tyndall to be mostly correct, in that scattering was responsible for the blue color of the sky; however, it was due to scattering by the atmospheric atoms themselves, rather than some contaminant particles as Tyndall had suggested.

Later observations by Gustav Mie (September 29, 1869, to February 13, 1957) quantified the principles concerning the scattering of light by larger spherical particles. For these systems, scattering is mostly governed by physical optics (refraction, reflection, absorption, etc.). These forces can affect each wavelength of light differently, and thus some chromatic alterations can be observed. Hues can be created depending on a range of factors including particle size, differences in refractive index between the molecule and the medium, as well as the wavelength and angle of incident light. A faded blue color can be generated in certain conditions; however, these systems typically consist of spherical, uniformly distributed particles around the size of the wavelength of visible light (400–800 nm).[10]

PHYSICAL AND BIOLOGIC PROPERTIES OF HYALURONIC ACID GEL FILLERS

HAs are large, high-molecular-weight molecules made up of repetitive sequences of 2 modified sugars. These chains can be many thousands of sugars long. They are naturally occurring in humans, and are perpetually subject to cycles of degradation and synthesis. To prevent physiologic enzymatic degradation, synthetic HA gels are extensively cross-linked by proprietary chemical processes. This cross-linking makes them resistant to native hyaluronidase activity and thus prolongs the duration of clinical action. Additionally, the type and amount of this cross-linking determines the physical and rheologic properties of a gel, which are often quoted in marketing materials as making one gel superior to another.[11]

Individual HA chains within this gel matrix can vary widely in size depending on the number of sugar repeats. Average chain lengths for commercially available fillers range from 300 μm to 700 μm[11]; however, a range of lengths are present in each available preparation. The diameter of any individual chain particle is in the range of 100 nm,[12] depending on conformation and folding.

Naturally occurring HA is not simply a structural protein; it is also a biologically active substance. It is known to be important in cell adhesion, chemotaxis, and differentiation. It is also involved in a range of physiologic processes including wound healing, fibroblast activation, and vasculogenesis.[13–15] The activity of naturally occurring HA is no doubt dependent on the signaling milieu in which it is found; however interestingly, differential physiologic action is also known to depend on the size and concentration of the hyaluron particles.[13] Whether synthetic HA gels are involved in these processes is not currently known; however, it could be reasonably postulated that HA fillers, whether in primary hydrogel format or after partial degradation, could have some bioactivity.

HYALURONIC ACID GEL FILLERS, LIGHT, AND THE TYNDALL EFFECT

With some basic understanding of the Tyndall effect and HA gel physical chemistry, it is possible to speculate on their interactions. Clearly, the Raleigh criterion cannot be applied to HA gels. Hyaluronic acid particles are far too large to have uniform electrical fields, being approximately 10× in diameter and 10,000× in length. Therefore, they will not be described by Raleigh's equations, and any blue hue could not be related to phenomenon similar to those creating a blue appearance of the sky.

They do however have a single dimension less than the wavelength of light (diameter <400 nm) and thus could potentially demonstrate Tyndall scattering. Even if possible, this scattering would not be described by the Mie solution, as they are non-spherical. Further, the particles are unequally distributed in hydrogel format and extensively cross-linked, making physical optics less likely to create a cohesive scattering system (for instance, preferentially scattering a specific wavelength of light). It may be expected that they would demonstrate interactions with light at the gel–air interface more akin to a solid polymer such as plastic (albeit, potentially including chromatic aberration). In terms of physical optics, it is unlikely that hues could be generated by HA gels, and if they were, such hues would not be explained by the Raleigh criterion, Tyndall effect or the Mie solution.

To test these theoretical determinations, some small demonstrations can be performed. By passing white light through a known colloidal substance and HA gel, respectively, the appearance of Tyndall scattering can be observed, or not, in both simultaneously. For instance, in Figure 1A white light is first passed through a colloidal solution (colloidal silver) and then through an aliquot of HA gel filler. The liquid clearly demonstrates Tyndall

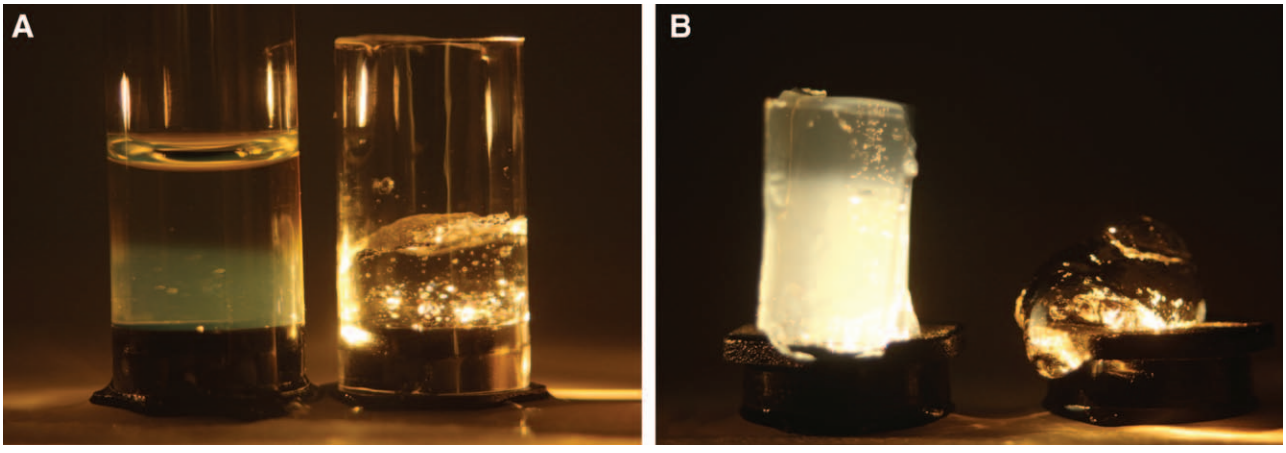

FIG. 1. **A**, White light passed through colloidal silver and hyaluronic acid gel, demonstrating Tyndall scattering with blue hue through the colloidal mixture and not through the hyaluronic acid gel. **B**, White light passed through solid state colloid (gelatin) and hyaluronic acid gel, demonstrating a Tyndall cone within the colloidal mixture and reflection and refraction through the hyaluronic acid gel.

scattering, with a blue hue, while the HA gel does not. This can also be replicated in the solid state, to avoid potential interference from the air–container interface. Again, in this case, Figure 1B demonstrates Tyndall scattering in the colloidal solid (gelatin), and its absence from the HA gel on the right, which tends to reflect at the air interface, rather than scatter within.

These simple experiments confirm the authors' theoretical assumption that HA gel fillers do not show Tyndall scattering, at least in air. It may be argued however that the HA gel is somehow optically different in the subdermal plane, and thus it may be possible that Tyndall scattering occurs in vivo. This too is highly unlikely when considering the optical properties of skin.

It is well established that skin preferentially transmits red light over blue light.[16] At the depth of subdermal HA, there is very little blue light content. Thus, only a small amplitude of blue light could be scattered in this plane in order to produce a dim hypothetical Tyndall cone. Further, should a Tyndall effect occur subdermally, the blue light "generated" would be filtered through the skin again as it passes to the observer, and thus would be unlikely to escape at sufficient intensity in order to be perceived.

These small experiments and theoretical considerations support the contention that HA gels do not demonstrate Tyndall scattering in vitro. Further if they were to scatter in vivo, the blue light amplitude of this scattering would be extremely low, and essentially completely filtered by the skin before an external observer could perceive it.

IF NOT TYNDALL THEN WHAT?

For all these reasons, it appears unlikely that the Tyndall effect accurately describes the blue hue of subdermal HA. The effect is potentially more accurately described by the second article quoted in the original Hirsch et al. article.[5] This referenced study was investigating the optical problem of why veins appear blue. The authors of that article described the phenomenon generally in terms of 3 factors: the reflective properties of the skin, the absorptive properties of blood, and the perceptive properties of the observer.[17]

As noted above, skin tends to transmit red light far more than blue light.[16] Thus, subdermal light is heavily skewed to the red spectra. This red light interacts with many optically active particles in the subdermal layers, particularly hemoglobin. Hemoglobin tends to absorb all wavelengths of light, and although blue light is much more strongly absorbed than red light, some red light is filtered through these interactions.[18]

The filtering activity of hemoglobin is physiologically concentrated within blood vessels; thus, light interacting with blood vessels would have a greater amount of filtering than light striking the surrounding structures (i.e., more light will be absorbed by blood vessels than the surrounding tissues). However, it is already established that skin and subdermal tissues specifically filter blue wavelengths of light (much of which is scattered or reflected at the air–skin interface). As a result of their subdermal location, the spectra of light striking blood vessels (and thus getting partially absorbed) are mostly in the red wavelengths.

As blue light is scattered superficially and red light is absorbed in the deeper planes, it creates a situation in which the light overlying a vein has slightly less red light intensity than the tissues surrounding it. Alternatively stated, the light over a vein has a relative red deficit.[17]

This red deficit is somewhat enhanced by the second aspect of this perceptual process, the deoxyhemoglobin content in veins. Venous blood contains a higher concentration of deoxyhemoglobin compared with arterial blood. Deoxyhemoglobin absorbs slightly more light overall (and thus more of red) than its oxygenated counterpart. All things being equal (depth, size, vessel wall thickness, etc.), the red deficit over a vein would be greater than over an artery. This difference contributes only a small amount to the overall effect,[17] which can be confirmed by observation of venous and arterial blood intraoperatively, where they only differ slightly in hue.

The final and most critical factor in this phenomenon is human color perception. Spectrally, the color reaching an observer's eye overlying a superficial vein is slightly gray relative to the surrounding tissues, having a small amount of red missing. However, it clearly appears blue to the observer. This "illusion" can be understood within the framework of a concept known as simultaneous contrast.

Although thought to have been observed by early thinkers including Aristotle and Leonardo Di Vinci, simultaneous contrast was operationalized by the French Chemist Michel Eugène Chevreul (August 31, 1786, to April 9, 1889) in his treaty The Principles of Harmony and Contrast of Colors and Their Applications to the Arts.[19]

Essentially, simultaneous contrast describes how the perception of a colored area is dependent on the surrounding colors in the visual scene. Thus, changing the context in which a wavelength spectrum is viewed will alter the viewer's perception of the light's color. These ideas have long been understood by the artistic community and are demonstrated in many famous works, for example the Haystack series by Claude Monet (November 14, 1840, to December 5, 1926).

How this applies to the problem of blue vein coloration is related to the red deficit occurring over a subdermal vein. For reasons described above, the specific area over a vein has relatively less red light content than all the surrounding tissues. Seen in the context of a more red heavy visual scene, the human perceptual system interprets this spectral alteration as the color blue.

This can be demonstrated dramatically in Figure 2. In this image, the pigment color of OU is precisely the same brown (CMYK: 49 62 58 28), and the burette in both figures also has the same spectral composition, in the blue range (CMYK: 62 26 32 1). When viewed in the context of a red hue overtone (Fig. 2A), the figure's OD clearly appears blue. The effect is dramatically revealed in the right panel where the color context is removed, and OU appear brown (Fig. 2B).

HOW IS HA GEL LIKE A VEIN?

The authors postulate that subdermally injected HA in certain patients can recreate the physiologic conditions in which red light is preferentially absorbed, and is thus perceived as being blue due to simultaneous contrast phenomena.

The biology of this optical arrangement remains unknown at present; however, a number of mechanisms could be proposed. The first series of explanations would contend that HA injection somehow alters the tissues physically and allows a vessel (or network of vessels) that normally does not receive red light to absorb it. This may be due to the addition of an optically clear substance between the skin surface and the vessels, so that red light can travel deeper and be partially absorbed. It may alternatively be possible that the tissues above and below the HA are compressed sufficiently that the amount of optically active tissue between the skin and the vessel is functionally reduced. A further theory could involve relative magnification of the subdermal vascular plexus by HA gel globules.

These series of postulates appear somewhat unlikely when a few observations are taken into account. The first is that the distribution of the blue hue tends to be stereotyped, and not confined to the specific areas where HA gel was directly injected (i.e., the effect is evenly spread, although not the gel).

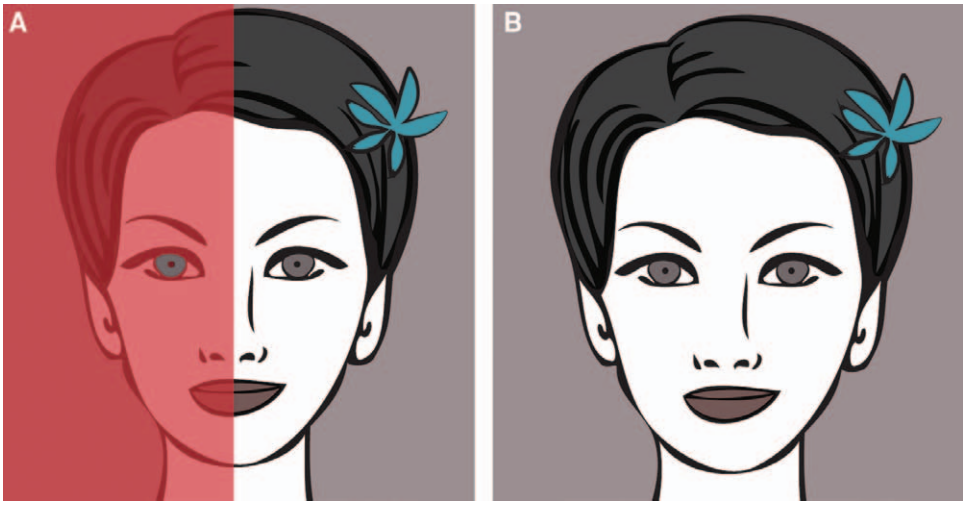

FIG. 2. Simultaneous contrast demonstration. The eye color is precisely the same for OU in each panel. The removal of red contextual color on the right (**A**) reveals the true brown–gray eye hue (**B**).

Additionally, the hue appears evenly distributed and is not more intense in areas with higher concentration of HA, and vice versa. Further, the effect is often delayed, suggesting that immediate physical optical phenomena are not responsible for the change. Finally, the intensity of the hue does not vary with the amount of HA gel injected, even from one side of the face to the other. For these reasons, it appears less likely that the optical properties of HA gel are involved in creating the blue hue.

Another group of explanations could relate to changes in local vasculature. For instance, an osmotic gradient created by the HA may lead to engorgement of local vessels making them larger and more likely to absorb red light. Alternatively, local compression of venous outflow could potentially lead to a similar engorgement, creating essentially the same physiologic effect. These are possible scenarios, but again less likely, as they do not occur immediately and do not demonstrate dose–response relationships.

A final series of hypotheses relate to the biologic activity of HA gels. Hyaluronic acid breakdown products and/or normal hyaluronidases concentrated in the region may be biologically actively producing changes in the local tissues, leading to the optical phenomena observed. This would be expected to be a regional effect, not confined to the specific area of injection. The stereotyped location and distribution of the hue could then be explained by graded local variability across the eyelid in the pigment content, thickness, and structural characteristics of dermal layers acting on the incident light.

These theories are of course speculative, and more work is required to elucidate the causes and possible treatments of the discoloration associated with HA injections. Overall, however, it is clear that the blue hue phenomenon after HA gel injection has been misinterpreted as being described by the Tyndall effect, and unfortunately this moniker has persisted. The authors contend that more appropriate terminology could simply reflect the physical findings and that the effect could be referred to as blue–gray dyschromia.

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